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CONTRIBUTION OF TRANS 3′-HYDROXYCOTININE AND GLUCURONIDE CONJUGATES OF NICOTINE METABOLITES TO THE MEASUREMENT OF COTININE BY RIA.

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Interlaboratory studies have shown that cotinine measured in urine by radioimmunoassay (RIA) generally gives results which are higher than those obtained by gas chromatography (GC) methods of analysis. However, cotinine standards added to urine results in complete recovery by both methods. Recent advances in nicotine metabolism have determined trans 3′-hydroxycotinine to be the major nicotine metabolite present in urine and have further identified glucuronide conjugates of nicotine, cotinine and 3′-hydroxycotinine. These newly found metabolites are not readily quantitated by GC methods of analysis and their contribution to immunoassay methods have not been determined. We hypothesized that the higher RIA results observed in comparison studies could arise from cross-reactivity with one or more of these metabolites. To test this hypothesis, we conducted a series of studies to characterize our antibody and to evaluate a spectrum of nicotine metabolites in the urine of smokers.

Authentic trans 3′-hydroxycotinine was added to urine obtained from non-smokers. Within the range of 1-300 ng/ml of added hydroxy-cotinine, the RIA assay measured approximately 35% of this nicotine metabolite. Urine samples obtained from smokers were then incubated with ß-glucuronidase for 6 hours at 37°C and the RIA cotinine levels were compared with those obtained on untreated samples from the same smokers. Results revealed no difference in metabolite detection between the two samples from each smoker. This suggests that glucuronide conjugates, if present, are detected by the RIA assay method.

Extralut 3 columns were used to extract ß-glucuronidase treated and untreated urine samples. With untreated urine, over 60% of the assayable material was retained by the column. However, when samples were incubated with ß-glucuronidase prior to column extraction, only 30% of the RIA reactive components were retained. Finally, when an inhibitor of ß-glucuronidase was added to the incubation mixture, the ß-glucuronidase effect was eliminated. These results suggest that in addition to holding back 3′-hydroxycotinine, glucuronide conjugates are retained by Extralut columns.

Overall, these results confirm our hypothesis and demonstrate that the RIA assay for urine cotinine provides measurement of free and conjugated cotinine as well as partial reactivity to 3′-hydroxy-cotinine. Experiments are being conducted to quantitate levels of individual metabolites and to explore inter-individual variation in conjugation of nicotine metabolites in urine.